

## ДОСВІД ОТРИМАННЯ ЗРІЛОЇ ІКРИ СИБІРСЬКОГО ОСЕТРА (*ACIPENSER VAERII* (BRANDT, 1869)) У НЕТРАДИЦІЙНІ РИБНИЦЬКІ СТРОКИ

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**Мета.** Дослідити особливості отримання овульованої ікри сибірського осетра в заводських умовах у нетрадиційний (зимовий) період та визначити основні рибницькі показники використаних в експериментах плідників.

**Методика.** Матеріалом для досліджень були самки сибірського осетра у віці 10+ масою тіла 6,20-8,24 кг, вирощені в умовах плавучих садків у водоймі з природним температурним режимом лісостепової зони України. Експерименти виконували за регульованого режиму температури води у третій декаді січня з використанням натуральних гонадотропних препаратів для стимулювання дозрівання статевих продуктів риб. У процесі підготовки плідників до проведення рибницьких робіт застосовували установки рециркуляційного водопостачання. Визначення рибницьких показників та дослідження фізико-хімічних чинників водного середовища здійснювали за загальноприйнятими методиками. Результати експериментів оцінювали статистично за допомогою стандартних програм.

**Результати.** За температури води 14–15°C, в залежності від дозувань гонадотропної речовини (2,5–4,5 мг/кг маси риб) зареєстровано різну ефективність дозрівання гонад в експериментальних групах плідників. Після застосування дозувань гонадотропного препарату 3,5 та 4,5 мг/кг дозріло понад 85% самок. У варіанті з мінімальною дозою гонадотропної речовини 2,5 мг/кг позитивно відреагувало на гормональну стимуляцію 57% самок. Середня маса статевих продуктів, отриманих в окремих групах плідників, становила 819,5–1079,0 г. Середні показники робочої плодючості та відносної робочої плодючості у дослідних групах самок сибірського осетра змінювались відповідно в межах 47,95–52,64 тис. ікринок та 6,93–7,17 тис. ікринок/кг маси риб. Тривалість дозрівання риб після другого (вирішального) введення гормонального препарату до моменту виникнення процесу овуляції яйцеклітин переважно змінювалась у межах 15-20 годин.

**Наукова новизна.** Досліджено особливості отримання зрілих статевих продуктів від самок сибірського осетра із застосуванням нових для аквакультури України методів заводського відтворення осетрових риб у нетрадиційний зимовий період.

**Практична значимість.** Результати досліджень можуть використовуватись для розвитку сучасних методів аквакультури осетрових риб в умовах господарств індустріального типу.

**Ключові слова:** плідники сибірського осетра, індустріальне рибництво, нетрадиційні технології, ікра, овуляція, рибницькі показники.



AN EXPERIENCE OF OBTAINING MATURE EGGS OF THE SIBERIAN STURGEON  
(*ACIPENSER BAERII* (BRANDT, 1869)) IN NON-TRADITIONAL PERIOD

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**Purpose.** To investigate the peculiarities of obtaining ovulated eggs of the Siberian sturgeon in hatchery conditions in non-traditional (winter) period and determine major features of brood fish used for the experiments.

**Methodology.** The material for the study were age-10+ Siberian sturgeon females with body weights of 6.20–8.24 kg grown in conditions of floating cages in a water body with natural temperature regime of the forest-steppe zone of Ukraine. The experiments were conducted with a regulated water temperature regime in the third decade of January with the use of natural gonadotropic drugs for the stimulation of egg maturation in fish. Brood fish were prepared for the experiment in the recirculating aquaculture system. Measurement of fisheries parameters and study of physicochemical factors of the aquatic medium were performed according to generally accepted methods. Experiment results were assessed statistically using standard software.

**Findings.** With water temperature of 14–15°C, depending on the dosage of the gonadotropic drug, different efficiencies of gonad maturation in experimental groups of fish were detected. More than 85% of females matured after dosing of gonadotropic drugs 3.5 and 4.5 mg / kg. In the version with a minimum dose of gonadotropic substance 2.5 mg / kg responded positively to hormonal stimulation 57% of females. The average weight of eggs obtained in some groups of brood fish was 819.5–1079.0 g. Average working fecundity and relative working fecundity in experimental groups of the Siberian sturgeon females varied within 47.95–52.64 thousand eggs and 6.93–7.17 thousand eggs/kg of fish body weight, respectively. The duration of maturation after the second (resolving) administration of the gonadotropic drug until the moment of the occurrence of the ovulation process varied mainly within 15-20 hours.

**Originality.** The peculiarities of obtaining mature eggs of the Siberian sturgeon with the use of methods of industrial reproduction of sturgeons, which are new for the aquaculture of Ukraine, were studied.

**Practical value.** The study results are of interest for the development of modern methods of sturgeon aquaculture in conditions of fish farms of industrial type.

**Key words:** brood Siberian sturgeon, industrial aquaculture, non-traditional technologies, eggs, ovulation, fisheries parameters.

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ОПЫТ ПОЛУЧЕНИЯ ЗРЕЛОЙ ИКРЫ СИБИРСКОГО ОСЕТРА (*ACIPENSER*  
*BAERII* (BRANDT, 1869)) В НЕТРАДИЦИОННЫЕ РЫБОВОДНЫЕ СРОКИ

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**Цель.** Исследовать особенности получения овулированной икры сибирского осетра в заводских условиях в нетрадиционный (зимний) периоды и определить основные рыбоводные показатели использованных в экспериментах производителей.

**Методика.** Материалом для исследований послужили самки сибирского осетра в возрасте 10+ с массой тела 6,20–8,24 кг, выращенные в условиях хозяйства индустриального



типа в водоеме с естественным температурным режимом лесостепной зоны Украины. Эксперименты выполняли при регулируемом режиме температуры воды в третьей декаде января с использованием натуральных гонадотропных препаратов для стимуляции созревания половых продуктов рыб. В процессе подготовки производителей к проведению рыбоводных работ применяли установки рециркуляционного водоснабжения. Определение рыбоводных показателей и исследование физико-химических факторов водной среды осуществляли по общепринятым методикам. Результаты экспериментов оценивались статистически с использованием стандартных программ.

**Результаты.** При температуре воды 14–15°C, в зависимости от дозировок гонадотропного вещества (2,5–4,5 мг/кг массы рыб), зарегистрирована различная эффективность созревания гонад в экспериментальных группах производителей. После применения дозировок гонадотропного препарата 3,5 и 4,5 мг/кг созрело с выше 85% самок. В варианте с минимальной дозой гонадотропного вещества 2,5 мг/кг положительно отреагировало на гормональную стимуляцию 57% самок. Средняя масса половых продуктов, полученных в отдельных группах производителей, составляла 819,5–1079,0 г. Средние показатели рабочей плодовитости и относительной рабочей плодовитости в экспериментальных группах самок сибирского осетра изменялись соответственно в пределах 47,95–52,64 тыс. икринок и 6,93–7,17 тыс. икринок/кг массы рыб. Продолжительность созревания рыб после второго (разрешающего) введения гормонального препарата до момента возникновения процесса овуляции яйцеклеток преимущественно изменялась в пределах 15–20 часов.

**Научная новизна.** Исследованы особенности получения зрелых половых продуктов от самок сибирского осетра с применением новых для аквакультуры Украины методов заводского воспроизводства осетровых рыб в нетрадиционный зимний период.

**Практическая значимость.** Результаты исследований могут использоваться для развития современных методов аквакультуры осетровых рыб в условиях хозяйств индустриального типа.

**Ключевые слова:** производители сибирского осетра, индустриальное рыбоводство, нетрадиционные технологии, икра, овуляция, рыбоводные показатели.

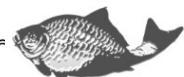
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## PROBLEM STATEMENT AND ANALYSIS OF LATEST ACHIEVEMENTS AND PUBLICATIONS

Due to the decrease in the number of sturgeon populations at their natural habitats, the main condition for the fish market saturation with a variety of sturgeon products should be the dynamic development of modern sturgeon-culture technologies [1–3].

In the list of perspective technological schemes of sturgeon aquaculture the greatest economic attractiveness is characterized by the caviar-commodity direction of exploitation of breeders herds formed in the conditions of the enterprises with a high level of fish farming intensification [4–6].

Among the members of the family *Acipenseridae* Siberian sturgeon has significant prospects for the development of caviar-commodity sturgeon culture. After all, it is characterized by a significant spread in intensive aquaculture farms and enjoys the constant attention of researchers due to its high resistance to various stressors in the process of artificial reproduction and cultivation in different technological schemes of industrial sturgeon farming [7–9]. At the same time, methods of using Siberian sturgeon breeders to obtain ovulated roe in non-traditional fish farming periods have not yet been sufficiently covered in the special literature and are new to aquaculture in Ukraine. This



in the first instance substantiates the relevance and novelty of the research.

### **HIGHLIGHT OF THE EARLIER UNRESOLVED PARTS OF THE GENERAL PROBLEM. AIM OF THE STUDY**

The bulk of the published materials on experimental researches conducted in Ukraine is devoted to works with breeding groups of sturgeon fish based at specialized sturgeon fish farms and in the conditions of separate factory breeders of full-system pond aquaculture farms. Selection of mature caviar from brood stock at these enterprises was carried out in the traditional for certain species of sturgeon breeding seasons in order to further grow viable young fish. However, in Ukraine still insufficiently analysed are the methods of operation with sturgeon breeding herds in controlled production of ovulated roe in different seasons of the year for the needs of the caviar-commodity development of sturgeon aquaculture. This primarily applies to sturgeon farms of the industrial type with the maintenance of repair and breeding groups of sturgeon fish in floating gardens, installed in reservoirs with natural temperature. In the past, the main species of sturgeon in experiments on such technological schemes of industrial aquaculture in Ukraine were sterlets [10, 11]. At the same time, the organization of food caviar production which uses other sturgeon aquaculture species, in particular Siberian sturgeon, can create a number of economic benefits, primarily in the quality of raw caviar [3, 8, 9].

In light of this, the aim of the research pursued was to determine the main fishpond indicators in experimental works on obtaining ovulated Siberian sturgeon caviar in non-traditional spawning periods using broodstock grown in floating gardens at natural water temperature of the Forest-Steppe of Ukraine.

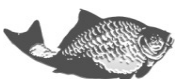
### **MATERIALS AND METHODS**

The collection of experimental materials was carried out during 2020–2021 on the basis of the fishery enterprise of industrial type LLC-SRP "Sturgeon", located in the Kiev region in the forest-steppe physical-geographical zone.

Mature 11-year-old Siberian sturgeon broods were used for the first time in studies to obtain mature sexual products.

Breeding and winter keeping of different age groups of Siberian sturgeon during the whole period of uterine herd formation was carried out in floating gardens at natural temperature on an artificial reservoir (in a flowing canal) with water supply from Kaniv Reservoir of the Dnipro Cascade. For this purpose, we used mesh cages with an area of 24 m<sup>2</sup> (4x6 m) with different mesh sizes in the case (depending on the fish size). The walls of the cages were deepened into the water by 2.5–3.0 m, which provided a useful volume of cage chambers on average up to 60–65 m<sup>3</sup>. The cages were placed on fixed pontoons. The cage line was installed above the depths of 5–9 m at a constant water exchange with a flow velocity of 0.05–0.20 m/sec. Fish farming, depending on age and body weight, was carried out with different planting densities, which mainly varied within 7–15 kg/m<sup>2</sup> of garden area. Throughout the growing period, Siberian sturgeon breeding groups were fed with specialized feeds of well-known Western European fish feed producers.

Work on artificial production of mature sexual products from sturgeon females was



performed in breeder equipped with recirculating water supply system with thermoregulation of the aquatic environment.

Obtention of Siberian sturgeon ovulated eggs was carried out in a non-traditional time for the reproduction of sturgeon fish (the third decade of January) with the use of intramuscular injections to broods with a suspension of dehydrated pituitary carp. Three total doses of gonadotropic substance were tested: 2.5; 3.5 and 4.5 mg / kg of fish weight used for hormonal stimulation, respectively, in the 1st, 2nd and 3rd experimental groups of sturgeon females, 7 individuals each. Two-times pituitary injections were given every 12 hours with the introduction of preliminary and decisive doses of the drug in a ratio of 1:9. Lifetime selection of mature sexual products was carried out by squeezing the eggs after trimming the oviducts of fish by the method of Podushka S. B. [12]. To determine the fertility of fish, the number of eggs per 1 g of caviar was calculated from samples of 3–5 g weight.

After the sturgeon broods were moved from the cage to the hatchery pools in the first half of January, a regulated increase in water temperature to the predicted “spawning” values (about 14°C) was used in the process of preparing the fish for ovulation. During the preparatory holding in pools of ICA type, the planting density of sturgeon females was up to 10–12 kg/m<sup>2</sup> at a constant water flow of up to 15 l/min.

In order to improve the control of individuals in the breeding herd, electronic tagging of fish was used, with identification of data using a scanner for microchips made in Germany (Planet ID). Ultrasound examination of fish using a portable ultrasonographic device made in China (Mindray DP-10 with a linear sensor 5-10 MHz) was used to determine the sex and stages of maturity of the gonads of Siberian sturgeon *in vivo*.

Experimental groups of broodstock to perform work on ovulating eggs were formed on a random basis from the total number of pre-selected sexually mature fish within one age group (10+).

Determination of fishpond parameters of experimental groups of sturgeon and physicochemical parameters of the aquatic environment was carried out using generally accepted methods in fish farming and hydrochemistry [13,14].

The obtained experimental results were evaluated statistically using standard programs [15].

## STUDY RESULTS AND THEIR DISCUSSION

Analyzing the results of hydrochemical studies of the reservoir in the area of the “Sturgeon” farm and comparing them with the existing regulatory requirements for sturgeon farming, it can be noted that the fluctuations of the detected values of chemical composition of water did not differ significantly from the normative values (Table 1).

The studied water was characterized by average level of mineralization with the sum of ions of 313.2–475.0 mg/dm<sup>3</sup> and according to the classification of O. O. Alyokin belonged to the hydrocarbonate class of the calcium group. The concentration of hydrocarbons in water ranged from 183.1 to 280.7 mg/dm<sup>3</sup> with a calcium ion content of 46.1 to 62.1 mg/dm<sup>3</sup>. The total water hardness was in the range of 4-5 mg-



eq/dm<sup>3</sup>. The values of the hydrogen index (pH) of water varied between 7.3 and 8.3 at the maximum values in the summer months. A certain exceedance of the normative values for the concentration of ammonium nitrogen with maximum values up to 1.49 mg N/dm<sup>3</sup> was registered. An increase of ammonium nitrogen in water was observed, in particular, in early spring during the period of intense snowmelt. However, this excess of normative values was short-lived. The concentration of nitrite nitrogen did not exceed 0.09 mg N/dm<sup>3</sup>, nitrate nitrogen - 1.37 mg N/dm<sup>3</sup>, which corresponds to existing standards. Mineral phosphorus and total iron were found in the amount approaching the normative, respectively, not more than 0.79 mg P/dm<sup>3</sup> and up to 0.97 mg Fe/dm<sup>3</sup>. The permanganate oxidation of water was from 7.6 to 15.3 mg O/dm<sup>3</sup>, which indicates the absence of excessive contamination of the reservoir with easily soluble organic matter. In general, the studied water in the given chemical composition can be considered typical for reservoirs of the region and suitable for reproduction and cultivation of sturgeon fish, which is confirmed by the results of fishery activities at the base enterprise LLC-SRP «Sturgeon».

Table 1. The results of hydrochemical research

No	Indicators and units of measurement	The value of the indicator (limits of fluctuations)	Regulatory values for sturgeon farms
1	pH	7,3–8,3	7,0–8,0
2	Free ammonia, NH <sub>3</sub> , mg N/dm <sup>3</sup>	0,004–0,06	until 0,05
3	Permanganate oxidation, mg/dm <sup>3</sup>	7,6–15,3	until 15,0
4	Ammonium nitrogen, NH <sub>4</sub> <sup>+</sup> , mg N/dm <sup>3</sup>	0,35–1,49	until 0,5
5	Nitrites, NO <sub>2</sub> <sup>-</sup> , mg N/dm <sup>3</sup>	0,06–0,09	until 0,1
6	Nitrates, NO <sub>3</sub> <sup>-</sup> , mg N/dm <sup>3</sup>	0,11–1,37	until 2,0
7	Mineral phosphorus, PO <sub>4</sub> <sup>3-</sup> , mg P/dm <sup>3</sup>	0,21–0,79	until 0,5
8	Total ferum, Fe <sup>+2+3</sup> , mg Fe/dm <sup>3</sup>	0,18–0,97	until 1,0
9	Total hardness, mg-eq/dm <sup>3</sup>	4,0–5,0	5,0–7,0
10	Mineralization, mg/dm <sup>3</sup>	313,2–475,0	until 2000,0

During 2020, which preceded the period of experimental work, the water temperature in the reservoir near the floating garden systems with Siberian sturgeon broods varied within 0.8–26.1°C. In summer, the average monthly values of water temperature in the studied areas of the reservoir were 20.6–23.9°C. Temperature maxima up to 25–26°C were recorded in certain periods of summer heat in the surface horizons of the water column.

Within the analysed period, in the area where tanks with the uterine material of Siberian sturgeon were placed, the content of dissolved oxygen in the water varied in the range of 4.7–10.5 mg O<sub>2</sub>/dm<sup>3</sup>. During experiments on obtaining mature sexual products of fish, the concentration of oxygen in the water entering the pools with Siberian sturgeon broods was 6.5–8.4 mg O<sub>2</sub>/dm<sup>3</sup>. That is, in all analysed cases, the oxygen regime of the aquatic environment of the experimental base mainly corresponded to favourable values for the life of sturgeon fish.



At the time of winter keeping of Siberian sturgeon brood stock in garden conditions, the water temperature dropped beyond 5°C in the second half of November, followed by its decrease during December to 1°C. At the beginning of the work on the preparation of sturgeon broods to obtain mature sexual products (first half of January), the water temperature in the gardens with the uterine material of sturgeon fish was within 1–2°C. After the sturgeon females were moved to the pools of the hatchery equipped with modern thermoregulatory means, a regulated (long-term) increase in the water temperature in the pools to 14°C was used to prepare the fish for the process of artificial ovulation.

The total dose of pituitary drug for Siberian sturgeon broods with two injections in different variants of the experiments ranged from 2.5 to 4.5 mg/kg of fish weight. Experimental doses of gonadotropic hormone were selected on the recommendations of the sturgeon sector of the Institute of Fisheries of NAAS, taking into account the experience of artificial reproduction of Siberian sturgeon in the usual spawning period. Preliminary injections were made at 14°C water. The injection of a decisive dose of gonadotropic substance in 12 hours after the first injection was made with water temperature rise by 1.0–1.5°C during the ripening period of the fish. The duration of offspring maturation after the decisive injection before ovulation of oocytes was 15–20 hours. With the above mentioned temperature, the entire period of maturation of sturgeon broods from the moment of previous injections to the beginning of the ovulation process varied in the range of 400–480 degrees-hours.

The individual weight of 11-year-old Siberian sturgeon females used in fish farming varied between 6.20 and 8.24 kg and averaged 7.09 kg. In the group of offspring №1 stimulated with a total dose of gonadotropic drug 2.5 mg/kg per pituitary stimulation, 57% of individuals responded positively, which can be considered an unsatisfactory result. Significantly higher efficiency of gonadal maturation was registered in fish of experimental groups №2 and №3 (85.7%) after the use of doses of gonadotropic substance 3.5 and 4.5 mg/kg. Fluctuations in the mass of selected sexual products in different sturgeon were in the range of 677–1552 g. Indicators of working fertility of fish varied from 40.36 to 71.39 thousand roe. The relative working fertility of brood stock ranged from 5.63 to 8.66 thousand roe / kg of fish weight (Table 2).

Analysing the data presented in table 2, it should be noted that, despite significant advantages in the efficiency of maturation of brood stock after administration of gonadotropic substances with doses of 3.5 and 4.5 mg/kg, other reproductive characteristics in all groups of fish were registered close to average indicators. Some advantages in terms of average weight of sexual products, gonadosomatic index and labour fertility in offspring with the lowest dose of gonadotropic hormone can be explained by the presence in this group of the largest female with a body weight of 8.24 kg, which responded well to pituitary all fish by weight of selected sexual products – 1552. At the same time, in the group of brood stock with the highest dose of gonadotropic drug, three smaller female sturgeons with a body weight of 6.46–6.88 kg were used, which were characterized by relatively low weight of selected caviar in the range of 677–736 g, which mainly affected on the reduction of the average gonadosomatic index and fertility in this group of fish.

Considering the conditions of sturgeon broods preparation for lifelong production



of mature sexual products in the non-traditional for the reproduction of sturgeon fish winter period, we should first highlight the process of adaptation of breeding stock to spawning water temperature. For this purpose, long-term "pre-spawning" keeping of brood stock should take place in the pools of the factory breeder with high efficiency of the thermoregulation system, which allows to provide the required temperature of the aquatic environment. At the same time, due to the relatively low intensity of metabolism in mature sturgeon, which are not fed during the incubation period in the pools of the hatchery, the consumption of oxygen in recirculation water is sufficiently compensated by aeration, and cleaning the environment from fish filter system.

**Table 2. The results of obtaining ovulated roe from the offspring of Siberian sturgeon**

Indicator	Weight of fish, kg	Mass of obtained sexual products, g *	Gonadosomatic index, % *	Working fertility, thousand roe*	Relative fertility, thousand eggs / kg of fish weight
Group of breeders № 1 (total dose of gonadotropic drug 2.5 mg/kg)					
M+m (n=4**)	7,41±0,31	1079,00±158,96	14,44±1,56	52,64±6,47	7,06±0,63
max	8,24	1552	18,83	71,39	8,66
min	6,82	866	11,50	42,43	5,63
Cv, %	8,50	29,47	21,66	24,58	17,74
Group of breeders № 2 (total dose of gonadotropic drug 3.5 mg/kg)					
M+m (n=6**)	7,06±0,24	900,67±75,56	12,73±0,87	50,58±3,12	7,17±0,37
max	7,76	1198	15,87	62,30	8,25
min	6,37	686	10,26	41,16	5,95
Cv, %	8,31	20,55	16,81	15,09	12,62
Group of breeders № 3 (total dose of gonadotropic drug 4.5 mg/kg)					
M+m (n=6**)	6,90±0,12	819,50±67,97	11,83±0,78	47,95±3,29	6,93±0,36
max	7,34	1110	15,12	62,16	8,47
min	6,46	677	9,84	40,36	6,10
Cv, %	4,36	20,32	16,17	16,83	12,66

Notes. \* Indicator values were determined after partial removal of ovarian fluid from the resulting roe.

\*\* Out of 7 sturgeon females, 4 fish responded to hormonal stimulation with a gonadotropic drug dose of 2.5 mg/kg. At other doses of gonadotropic drug from 7 broodstock in groups, 6 fish in each group responded to hormonal stimulation.

## CONCLUSION AND PERSPECTIVES OF FURTHER DEVELOPMENT

Estimation of reproductive indicators of Siberian sturgeon females in experiments on artificial production of mature sexual products in non-traditional winter terms generally indicates sufficient efficiency of fish farming. As a result of direct influence of ecological and physiological factors' complex, first of all regulated temperature mode in the conditions of recirculated water supply, in various experimental groups of sturgeon breeders with average weight of 6,90–7,41 kg indicators of working fertility





averaged 47,95–52,64 thousand roe at average values of relative working fertility 6,93–7,17 thousand roe/kg of fish weight. Higher maturation efficiency of Siberian sturgeon females (over 85%) was registered in experimental variants using dehydrated gonadotropic substances of dehydrated carp pituitary glands to stimulate maturation of eggs with total doses of 3.5 and 4.5 mg/kg of fish weight by double injections with distribution of previous and crucial dosages of gonadotropic drug in a ratio of 1:9.

The obtained results confirmed the prospects of the tested technological solutions in industrial fish farming, which are not widespread for the aquaculture of Ukraine, and which can be used for the effective development of the caviar-commodity direction of sturgeon farming.

For the further development of research in this area, it's worth to determine the effectiveness of other gonadotropic substances to stimulate the maturation of Siberian sturgeon gonads in non-traditional fish seasons, in particular to reduce the cost of purchasing appropriate hormonal drugs.

As to challenging targets for the scientific support of this problem, worthy of separate attention is the issue of comprehensive genetic monitoring of sturgeon breeding herds in order to improve the state of breeding resources of domestic sturgeon. To start with, this applies to representatives of a number of sturgeons imported to Ukraine from other centres of sturgeon aquaculture, one of which is the Siberian sturgeon.

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